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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/074,041	02/14/2002	Hideki Ishihara	0397-0440P	6640

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BIRCH STEWART KOLASCH & BIRCH
PO BOX 747
FALLS CHURCH, VA 22040-0747

EXAMINER	
GODDARD, LAURA B	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 06/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/074,041	ISHIHARA ET AL.	
	Examiner	Art Unit	
	Laura B. Goddard, Ph.D.	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 February 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-7 and 10-14* is/are pending in the application.

4a) Of the above claim(s) *7* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6 and 10-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. The request for continued examination (RCE) mailed April 18, 2006 has been acknowledged and the amendments mailed February 8, 2006 have been entered.

Claims 1-7, and 10 are pending. New claims 11-14 were added. Claims 7 is withdrawn from consideration as drawn to a non-elected species. Claims 1-6 and 10-14 are currently under prosecution.

Priority

2. Acknowledgment is made of Applicant's claim for foreign priority based on the specification, page 1. It is noted, however, that Applicant has not filed a certified copy of the Japanese Patent Application No. 2001-37115 as required by 35 U.S.C. 119(b). It appears that Applicant submitted a copy of the Japanese Patent Application at the time of filing (2/14/02), however, it does not appear to be a certified copy.

Claim Objections

3. Claim 12 is objected to because of the following informalities: The claim recites "poly(vinylidene fluoride)" and the parenthesis appear to be grammatically incorrect. The PVDF membrane is referred to as **polyvinylidene fluoride.** Appropriate correction is required.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. Claims 1, 2, 3, 6, and 10 rejected under 35 U.S.C. 103(a) as being unpatentable over Pan et al. (*J Biol Chem*, 1993, Vol. 268: 20443-20451) in view of Blain et al (Journal of Biological Chemistry, 1997, 272:25863-25872), Jeong and Nikiforv (*BioTechniques*, 1999, Vol. 27: 1232-1238, IDS), Facemyer and Cremo (*Bioconjug Chem*, 1992, Vol 3: 408-413, IDS), and Gray et al (US Patent 6,255,485, issued 7/3/2001, filed 8/6/1998).

The claims are drawn to a method for calculating the activity of a cyclin-dependent kinase (CDK) in a sample prepared from a living cell, comprising catching the cyclin-dependent kinase by an anti-cyclin-dependent kinase antibody, reacting ATP-γS with a substrate (that does not contain a sulfur atom) for a CDK, placing the reacted substrate on a membrane, coupling a labeling fluorophore or enzyme with a sulfur atom of the introduced monothiophosphate group of the substrate on the membrane, washing the membrane to remove excess label not coupled to the substrate, measuring the fluorescence from the label in the product or reacting the labeling enzyme with a substance to generate an optically detectable product and measuring the amount of the generated product, and calculating the activity of the CDK from the measured amount of label in the product with reference to a pre-produced curve (claims 1), or obtaining the

activity of the CDK from the measured amount of fluoresence or the measured amount of the generated product (claim 10), CDK1 (claim 2 and 6), fluorescent dye (claim 3), a histone H1 substrate (claim 6).

Pan et al. teach a method for calculating or obtaining the activity of cdc2 (CDK1) prepared from a cell sample comprising incubating a CDK1/cyclin complex with [γ-^{32}]ATP and histone H1 and measuring CDK1 phosphorylation activity by quantifying [^{32}P] in the product and comparing it to control measurements (page 20444, col. 1). Pan et al. does not teach reacting ATP-γS with a substrate, catching CDK with an antibody, labeling the substrate with a fluorophore, placing the substrate on a membrane, washing the membrane, and calculating the fluorescence from a labeled substrate.

Blain et al teach catching a cyclin-dependent kinase using an anti-cyclin-dependent kinase antibody (immunoprecipitation) for a kinase assay (p. 25864, col. 1).

Jeong and Nikiforv (herein referred to as "Jeong") teach a non-radioactive method of calculating protein kinase activity comprising reacting ATP-γS with a substrate, kemptide (which does not naturally contain a thiol group or sulfur atom), to create a thiophosphorylated product (page 1232, column 3) and measuring fluorescence values in the final product (page 1233, columns 1 and 2). The reference teaches the conventional wash step of removing excess label (p. 1232, col. 2). The reference teaches that the biggest drawback of the present method is the relatively slow rate of the biotinylation step, however this can be overcome by various methods and thus it represents a viable alternative to existing methods of screening protein kinases (page 1238, column 2). The reference suggests this method is useful for a wide range

of different kinases (page 1232, third column). Further, the reference teaches that presented is an alternative approach for detecting kinase activity wherein the method does not require the use of radioactivity and allows flexibility in the detection scheme (p.1238, column 2).

Facemyer and Cremo teach a method of using a protein kinase and ATP-γS to create a thiophosphorylated protein and the method of labeling a thiophosphorylated protein by coupling the sulfur of the protein phosphorothioate to a fluorescent haloacetate (page 409). It is noted that the reference further teaches that thiol groups in the substrate are blocked prior to reaction with ATP-γS (See Fig. 1).

Gray et al teach a CDK protein kinase assay wherein the CDK is reacted with substrate H1 and [γ-^{32}]ATP, loaded on a nitrocellulose membrane, the membrane is washed, and the labeled substrate is measured (col. 37 and 38; Example 7).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the methods of Blain et al and Jeong for the method of Pan et al. to assay CDK1 activity with a histone H1 substrate because Blain et al teach the conventionally used method of catching a cyclin-dependent kinase with an antibody for a kinase assay and Jeong specifically teach the disadvantages of traditional assays of enzyme activity of protein kinases which use [γ-^{32}]ATP which require radioactivity and multiple steps. One would have been motivated to substitute the methods of Blain et al and Jeong for the method of Pan et al. to assay CDK1 activity with a histone H1 substrate in order isolate the cyclin-dependent kinase from the cell sample to reduce artifacts from other CDKs and to eliminate the disadvantages

specifically taught by Jeong, and because Jeong specifically suggest that the method is useful for a wide range of different kinases.

Further, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention to substitute the direct fluorescent labeling of the thiol of the reacted ATP-γS of Facemyer and Cremo for the labeling steps of the combined references because Jeong specifically teach that the biggest drawback of their method is the relatively slow rate of the biotinylation step. One would have been motivated to substitute the direct fluorescent labeling of the thiol of the reacted ATP-γS of the Facemyer and Cremo for the labeling steps of the combined references in order to save not only time, but also the cost of the labeling reagents of Jeong.

Finally, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention to add the step placing the substrate on a membrane and washing the membrane to remove excess label because Gray et al expressly teach this step in a CDK assay. One would have been motivated to place the labeled substrate on a membrane and to wash the membrane in order to isolate the labeled product for measurement in a kinase assay as taught by Gray et al, and because the practice of using nitrocellulose membranes to isolate products for measurement is conventional practice and well-known in the art.

5. Claims 4, 5, 13 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pan et al, Blain et al, Jeong and Nikiforv, Facemyer and Cremo, and

Gray et al, (referred to as "the combined references") in further view of Abo et al (US Patent 5,518,911, issued 5/21/1996).

The claims are drawn to the method of claim 3 wherein the fluorescent dye is FITC (claim 4), the method of claim 1 wherein the labeling enzyme is peroxidase (claim 5), the method of claim 1 further comprising the step of blocking the membrane after the step of placing the substrate on the membrane (claim 13), wherein the membrane is blocked by an albumin (claim 14).

Pan et al, Blain et al, Jeong and Nikiforv, Facemyer and Cremo, and Gray et al, teach a method for calculating the activity of a CDK as set forth above. However, the combined references do not specifically teach labeling the substrate with FITC or peroxidase, or blocking the membrane with albumin.

Abo et al teach a kinase assay of calculating kinase activity of kinase prepared from a cell sample comprising immobilizing (or "catching") the kinase using an antibody (col.27, lines 14-20 and 63-67 to col. 28, lines 1-5; col. 29, lines 60-67 to col. 30, lines 1-5), reacting the kinase with the substrate in the presence of GTPγS (col. 30, lines 16-19; col. 45, Example 14), labeling the substrate with an enzymatic or fluorescent label (col. 28, lines 27-67; col. 29, lines 33-36), blocking the reaction by adding albumin (col. 29, lines 20-59), washing excess label from the reaction, and measuring the amount of labeled product (col. 29, lines 24-40). Abo et al teach that methods of labeling are conventional and known in the art (col. 12, lines 46-47; col. 28, lines 38) and specifically teach the use of FITC and peroxidase as labels (col. 12, lines 50-52). Abo et al teach

that albumin is a blocking agent or non-labeled competitor protein that is included to inhibit nonspecific binding (col. 29, lines 24 and 41-59).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to label the CDK substrate in the method taught by the combined references with a FITC or peroxidase as taught by Abo et al because Abo et al teach that these methods of labeling are conventional and known in the art. One would have been motivated to label the substrate in the method taught by the combined references using FITC or peroxidase in order to detect the substrate and measure kinase activity. Further, one would have been motivated to substitute the FITC or peroxidase label for the radioactive [^{32}P] label in the method of the combined references because FITC and peroxidase labels offer a safe method for labeling and detecting specific proteins in a sample without the use of hazardous materials such as radioisotopes.

Further, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to add albumin as a blocking agent in the method taught by the combined references because Abo et al teaches the method of blocking a reacting using albumin specifically for a kinase assay. One would have been motivated to add albumin as a blocking agent to the method taught by the combined references because albumin is a conventional blocking agent or non-labeled competitor protein that is included to inhibit nonspecific binding, hence increasing the specificity of the kinase assay.

6. Claims 11 and 12 rejected under 35 U.S.C. 103(a) as being unpatentable over Pan et al, Jeong, Blain et al, Facemyer and Cremo, and Gray et al, (referred to as "the combined references") in further view of Gopalakrishna et al (Analytical Biochemistry, 1992, 206:24-35).

The claims are drawn to the method of claim 1 wherein the membrane comprises a hydrophobic part (claim 11), wherein the membrane comprises polyvinylidene fluoride (PVDF) (claim 12).

Pan et al, Blain et al, Jeong and Nikiforv, Facemyer and Cremo, and Gray et al, teach a method for calculating the activity of a CDK as set forth above. However, the combined references do not specifically teach a method wherein the membrane comprises a hydrophobic part or wherein the membrane comprises polyvinylidene fluoride (PVDF).

Gopalakrishna et al teach a conventional approach in a kinase assay to determine kinase activity by preparing kinase from a cell sample, reacting the kinase with histone H1 and [^{32}P]ATP, placing the reaction on a PVDF membrane, washing the membrane, and measuring the labeled substrate to calculate kinase activity (p. 25, col. 1 and 2; p. 26, col. 1; abstract). Gopalakrishna et al teach that the PVDF filters have low nonspecific binding for proteins and at least a fourfold increase sensitivity for reliable estimation (p. 25, col. 1). PVDF is a hydrophobic polymer as evidenced by Pall Corporation (see "PVDF membranes for Western Transfer and Sequencing", p. 1).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to substitute the PVDF membrane for the nitrocellulose

membrane in the method taught by the combined references because Gopalakrishna et al teach the use of a PVDF membrane specifically for assaying kinase activity. One would have been motivated to substitute the PVDF membrane in the method taught by the combined references because of the PVDF membrane's low non-specific binding of proteins and increase in sensitivity for more reliable estimations of kinase activity.

7. **Conclusion:** No claim is allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura B. Goddard, Ph.D. whose telephone number is (571) 272-8788. The examiner can normally be reached on 8:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew can be reached on 571-272-0787. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura B Goddard, Ph.D.
Examiner
Art Unit 1642

JEFFREY SIEW
SUPERVISORY PATENT EXAMINER